injury in normolipemic swine. Formalin fixed, paraffin embedded coronary artery sections were stained using conventional ABC methodology and immunoperoxidase techniques. Human melanoma and normal kidney served as positive controls. Deletion and substitution controls for primary antibody were negative. Staining of contiguous sections with specific cell marker antibodies identified SMC, EC, macrophages (*MAC*) and myofibroblasts (*MYF*). $\alpha_v\beta_3$ staining was ordinally graded from 0 (none) to 3 (strong). *Results:* In the uninjured coronary artery, only the luminal endothelium expressed $\alpha_v\beta_3$. Marked temporal-spatial variation in $\alpha_v\beta_3$ expression was noted in all vessel wall locations, colocalizing with platelet thrombus (≥24 hours), MF, SMC, EC and to a lesser degree MAC (see Table).

Location	7 days	14 days	20 days	3 months
Neointima *(EC, SMC)*	2	3	2	1
Media *(SMC)*	3	2	1	0
Adventitia *(VV, MF, MAC)*	3	2	2	1

Conclusions: Following deep arterial injury, temporal $\alpha_v\beta_3$ upregulation by *SMC, EC,* and *MF* peaks at 7 days in the media and adventitia, and at 14 days within the neointima. Following injury, $\alpha_v\beta_3$ is also upregulated within the luminal EC and the medial SMC of the vasa vasora (*VV*). Temporal $\alpha_v\beta_3$ upregulation offers a mechanism for SMC, EC, and MF recruitment into regions of restenotic hyperplasia. Sustained $\alpha_v\beta_3$ integrin blockade may thus offer a cogent strategy to limit restenosis.

960-111 Modulation of Platelet Neutrophil Interaction with Pharmacological Inhibition of Fibrinogen Binding to Platelet GPIIb/IIIa Receptor

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This study was designed to investigate whether the inhibition of fibrinogen binding to the platelet membrane receptor GPIIb/IIIa (PL-Fb) could interfere with neutrophil-platelet adhesion (PL-Ne). Citrated whole blood obtained from 30 pts with unstable angina before and 4 to 12 hours after tirofiban (Tir, 0.1–0.15 μg/kg/min) was studied using 3 color whole blood flowcytometry. The leukocyte population was defined with a nucleic acid dye (LDS-751) and a monoclonal antibody (MAb), anti-CD45-PE, and the neutrophil subpopulation gated with cell size and granularity. PL-Ne was quantified with MAb anti-CD42a-FITC, as mean FITC intensity per neutrophil (PL-FI). FITC-labeled fibrinogen was used to detect platelet-fibrinogen binding, as % FITC-positive platelets (%PL-Fb). TRAP 5 μM, ADP 5 μM, and ADP plus epinephrine 1 μM (ADP + E) were used as agonists. Tir did not prevent platelet P-selectin expression, but decreased the number of platelets bound per neutrophil in basal state and following agonists stimulation (Table, x ± SD, *p < 0.05, #p < 0.01 vs pre-Tir). The decrease significantly correlated with the inhibition of fibrinogen binding to platelets (r = 0.75).

	Pre-Tir		Tir	
	PL-FI	% PL-Fb	PL-FI	% PL-Fb
Basal	3.0 ± 1.4	3.4 ± 2.5	2.2 ± 0.5*	0.6 ± 0.4#
ADP	5.5 ± 1.4	86.7 ± 9.2	3.1 ± 0.8#	9.0 ± 8.1#
TRAP	10.3 ± 2.4	88.5 ± 4.8	6.4 ± 2.0#	40.8 ± 30#
ADP + E	7.0 ± 2.8	94.5 ± 3.0	3.4 ± 0.9#	22.3 ± 21#

GPIIb/IIIa blockade reduces platelet-neutrophil binding; this may prevent mass accumulation of platelet and/or leukocyte on injured endothelium.

960-112 Intranasal Antiplatelet/Antithrombotic Efficacy of the Platelet GPIIb/IIIa Antagonist, DMP754

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DMP754 after its conversion to the free acid form, XV459 is a potent and specific platelet GPIIb/IIIa receptor antagonist. The present study was undertaken to determine its intranasal versus intravenous and oral antiplatelet/antithrombotic efficacy in dogs. Either DMP754 or its free acid form, XP280 demonstrated maximal and comparable antiplatelet efficacy at 0.025–0.1 mg/kg, IV or intranasal in mongrel dogs. The antiplatelet efficacy of DMP754 at 0.1 mg/kg, intranasal or IV was determined in a cross-over design (n = 8 in each group). In this study, a comparable and maximal antiplatelet efficacy for DMP754 after intranasal or IV was demonstrated with 97 ± 68% intranasal bioavailability. DMP754 administered at 0.1 mg/kg, intranasally or IV and at 0.3 mg/kg, PO prevented the incidence of electrolytic injury-induced arterial thrombosis in the carotid artery and prevented the incidence of cyclic flow reduction (CFR) in mechanically injured and stenosed femoral artery. Additionally, DMP754 at the above dose regimens resulted in significant reduction of electrolytic injury-induced femoral vein thrombosis. These data indicate that DMP754 has a comparable intranasal and intravenous antiplatelet and antithrombotic profile along with a significant improvement over its oral antiplatelet/antithrombotic profile. These data suggest the potential utility of intranasal DMP754 in various acute and chronic thromboembolic disorders.

960-113 Moesin: A Cell Membrane Protein Regulating Receptor-Cytoskeleton Interactions in Human Platelets

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Following various stimuli, platelet activation goes along with rapid shape change from a discoid biconcave form to a phenotype with multiple protrusions. This shape change and the reorganisation of the cytoskeleton favors cell-cell and cell-matrix interactions. Moesin, a 78 kDa protein, is a member of the ezrin, radixin and moesin (ERM) protein family and there is evidence that they interact with both cytoskeletal and membrane components. In a previous study, moesin was purified to homogenity from human platelet lysate and specific antibodies were elicited in chicken.

To study moesin-cytoskeleton interactions the cytoskeleton of resting and activated platelets was isolated by triton X 100 lysis and centrifugation resulting in a pellet fraction containing cytoskeletal proteins (15,000 × g: actin based cytoskeleton; 100,000 × g: membrane cytoskeleton) and a supernatant consisting of soluble not cytoskeletal bound proteins. Western blot analysis with a moesin specific antibody demonstrated the presence of moesin in the 15,000 × g pellet fraction only in thrombin activated platelets. In contrast, in the 100,000 × g pellet fraction moesin was found in resting and activated platelets with the majority of this protein remaining in the soluble fraction. This finding implies, that an association of moesin with the filamentous actin occurs, while most of the protein is associated with the membrane cytoskeleton or soluble during activation of platelets.

For further analysis of moesin interactions, platelet plasma membranes were isolated by glycerol lysis and an sucrose step gradient at 63,000 × g. Immunoblotting of the plasma membrane fraction revealed the presence of moesin despite some bands of slower motility then moesin. Immunoprecipitation with polyclonal moesin antibody of platelet plasma membrane fraction and whole RIPA buffer lysed platelets showed the presence of at least 3 potential binding proteins. These data indicate that moesin functions as a transmitter between the cytoskeleton and membrane components in platelets.

961 Regulation of Vascular-Cellular Growth and Function

Monday, March 17, 1997, 3:00 p.m.–5:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

961-116 The Pericardium as a Repository for an Angiogenic Growth Factor: Effect of Intrapericardial Basic Fibroblast Growth Factor on Myocardial Collateral Development

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We have previously shown that basic fibroblast growth factor (bFGF) enhances myocardial collateral development in dogs when administered via a systemic arterial or intracoronary (IC) route. In this study, we assessed the efficacy of repeated intrapericardial (IP) bFGF delivery, with collateral perfusion as the primary endpoint. We hypothesized that the pericardium could act as a local repository for bFGF, exposing epicardial collaterals to a relatively high bFGF concentration. Such a route of delivery might decrease the minimum effective bFGF dose, and obviate the need for repeated IC injections. Fifteen dogs underwent placement of ameroid constrictors on the left circumflex coronary artery (LCx) and implantation of indwelling IP catheters. Dogs were randomized to receive bFGF 2 mg/d × 7 days or vehicle during the period 10–16 days after ameroid placement, an optimum treatment interval based on our previous studies using other routes of bFGF delivery. Radiolabeled microspheres were used to assess collateral perfusion in the conscious state during maximal coronary vasodilatation 38 days after ameroid placement. Maximal LCx territory perfusion was 2.02 ± 0.24 ml/min/g in bFGF-treated dogs and 1.94 ± 0.16 ml/min/g in controls, with LCx/normal zone perfusion ratios of 0.39 ± 0.03 and 0.37 ± 0.03, respectively, (P = NS). Thus, in

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